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This case-control study examines breast cancer risk in relation to lifetime alcohol consumption. Subjects are 335 white and 35 black pre-menopausal and 900 white and 80 black post-menopausal women, age 35-79, from Erie and Niagara counties in western New York State, with incident, pathologically confirmed breast cancer. Controls are randomly selected and are frequency matched to cases on age, race and county of residence. Subjects receive a computerized interview which focuses on aspects of alcohol consumption such as frequency, quantity per occasion, beverage type, and drinking pattern through the lifespan. Important potential confounding factors such as reproductive and medical history, family history of disease, diet, exercise, smoking, passive smoking, occupation, residential history, and anthropometric measures and demographics are also assessed. A specimen bank is used to store biological samples for future research in relation to serum and urinary markers including hormones in serum, hormone metabolites in urine, serum and erythrocyte folate levels, blood levels of antioxidants, blood measures of oxidative status, and genetic polymorphisms in carcinogen metabolism. This study provides an important opportunity for an efficient examination of alcohol and other risk factors, particularly genetic variability, in relation to breast cancer risk, with potential for clarification of a significant public health problem.

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FOREWORD

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INTRODUCTION

This research is an epidemiologic investigation into the role of lifetime alcohol exposure in breast cancer etiology, research of considerable relevance to the issue of breast cancer prevention, providing insight on the role of a modifiable, and common exposure. It is being conducted in conjunction with two existing case-control studies as part of The Center for Clinical and Medical Epidemiology of Alcohol. These studies share the same protocol and control series.

The primary purpose of this study is to examine the history of alcohol consumption from adolescence through adulthood as a risk factor for pre- and post-menopausal breast cancer in women. We will also examine the possible role of factors such as genetic factors, estrogen receptor status, histology, and use of estrogen replacement therapy among post-menopausal women in mediating the effect of alcohol on breast cancer risk.

In this case-control study, women age 35-79 from Erie and Niagara counties in western New York with incident, pathologically confirmed cases of breast cancer are being interviewed (335 white and 35 African American pre-menopausal and 900 white and 80 African American post-menopausal women). Controls are women interviewed as part of the concurrently conducted, previously funded, case-control studies. Controls are randomly selected, those under age 65 from lists provided by the New York State Department of Motor Vehicles, those age 65 and over from enrollment lists of the Health Care Finance Administration. Controls will be frequency matched to cases on age, race, and county. Blood samples are stored in a biological specimen bank for future research.

Previous research in this area has relied on relatively crude measures of alcohol consumption, generally not distinguishing infrequent drinkers of larger amounts from individuals who drink smaller quantities more often, and those who drink with meals from those who do not drink with meals. There is some evidence that age when drinking began may also affect risk; studies have not examined in detail characteristics of early drinking. Further, there is some evidence that women may differ in their metabolic response to alcohol depending on genetic polymorphisms for enzymes involved in alcohol metabolism. Additionally, there is evidence that risk from alcohol may differ among other subgroups of women, such as by breast cancer estrogen receptor status and histology, and by use of exogenous estrogens among post-menopausal women.

BODY OF REPORT

During this first budget year, study protocols were developed and copies were submitted to 11 area hospitals for review by their Institutional Review Boards. As of this date we have confirmed written approval from ten of the hospitals. Approval from the remaining hospital is anticipated. We are working closely with four breast surgeons who see about 60% of women in this region with newly diagnosed breast cancer. All are cooperating fully with the study. Contact with other physicians is underway.

In the Statement of Work (Task 1), we had estimated that we would require three months to complete the IRB approval process. In fact, this process took almost a full year. In part, the delay was related to issues of scheduling IRB meetings by hospitals. There were also some difficulties in obtaining approval because of restrictions in the consent form imposed by the Army. Those difficulties have been resolved with the hospitals.

During this period, we also collaborated with several other scientists and physicians such that the study will be as inclusive as possible. We finalized all arrangements for interviewing as listed in the Statement of Work (Tasks 2 and 3) and have begun interviewing cases. We are continuing to enroll controls as part of the already existing case-control studies. We hired a Project Coordinator, a part time data manager, an administrative assistant, two interviewers, and a breast cancer survivor to do recruiting. We have developed a poster and brochures to be distributed to doctors' offices and mammography centers to support our recruitment efforts. We are developing consulting relationships and partnerships with various community groups including the Breast Cancer Network and the American Cancer Society.

During August we provided training for all interview staff. Hard copies of the questionnaire were provided, interview techniques for standardization were reviewed and computer training provided. In August we interviewed our first breast cancer case. Minor revisions in the computerized questionnaire were required and have been made. In accordance with the Statement of Work (Task 4), preparations for the ongoing data entry of the interview, maintenance of files from the computer-assisted interview, and entry of data from the sections of the interview completed by hand by the participant were developed and coincide with procedures used in the existing case-control studies.

During the summer, two nurse casefinders began working with hospital personnel in pathology and medical records departments in which we had IRB approval to establish methods of identifying eligible breast cancer patients. We set March 1, 1997 as the start date of diagnosis for inclusion of breast cancer patients in the study. When the nurse casefinders obtain the names of individuals aged 35-79 with a histologically-confirmed diagnosis of primary breast cancer, we mail a request to the physician for permission to interview the patient. Once approval is obtained, we invite the patient to participate. After securing the patient's written consent for the interview and optional blood draw, we proceed with the actual interview process. So far, we have identified 168 breast cancer cases and we have received physician approval to contact 57 of them. Seven women have refused to participate. To date, we have interviewed a total of 11

breast cancer cases. All the women interviewed have also consented to the blood draw. For cases who have been interviewed we are collecting information on stage of the cancer and other relevant characteristics of the tumor from hospital pathology records.

During this year we finalized procedures for the ongoing maintenance of the biological specimen bank, processing of samples for immediate determinations and for storage, tracking of samples and mapping of the freezer as outlined in the Statement of Work (Task 5). In terms of standardization of specimen collection, all blood is drawn at the same time of the day (7:00AM-9:00AM). For pre-menopausal women, blood drawings are scheduled for the luteal phase of the cycle to reduce, to the extent possible, variation in hormone levels related to the menstrual cycle. The time of the blood draw is recorded for assessment of any variation in blood markers related to the time of the draw.

Three different reliability studies are being conducted as part of the quality control measures for the specimen bank.

Biological Variability of Oxidation Parameters and Sex Hormones. Sex hormones and oxidation have been hypothesized to have a relationship to breast cancer. The purpose of this study is to find reliable biomarkers of sex hormones and of oxidation in healthy adults.

The goal is to characterize the intra-individual variability in oxidation parameters and reproductive hormones over a six month period. Seven pre-menopausal women and three men had their blood drawn once a month for six months. All the blood drawings were performed on the same day of the menstrual cycle for the women and in the same hour of the day after a 12-hour fast for all participants.

Intra-individual differences in urine hormone levels among the women have been examined. These data suggest that urine estrone-3 glucuronide may be useful as a biomarker of estrogenic pattern (1). Intra-individual differences in serum hormone levels will be analyzed at a later time.

Effect of Transportation on Oxidative Stress Indicators and Vitamins Determinated in Serum and in Plasma Most cases and controls have their specimens collected at our clinic in the Center for Preventive Medicine, close to the laboratories where the blood is processed. However, some participants recruited for the study have their blood drawn during home visits. This corollary study was designed to detect possible systematic effects on oxidative stress baseline determinations between blood samples drawn at the Center and blood samples drawn during home visits.

Subjects were 52 men recruited from personnel of the Department of Social and Preventive Medicine. Two similar sets of blood samples were collected from each at the Center for Preventive Medicine. The first one was handled as are blood samples normally drawn at the Center for Preventive Medicine and sent immediately to the laboratory for processing and storage. The second set was handled as are blood samples drawn at home visits. After drawing, the samples were transported for one hour by car to simulate the transportation from the home visit to the Center for Preventive Medicine. One hour is the average observed time frame for the

home visits between the time of blood drawing and the delivery to the laboratory for the usual blood process and storage. Differences in laboratory results between the two methods are currently being analyzed.

Effects of Long-Term Storage at both -196°C and -80°C on Biological Parameters. A protocol has been written for this study and is ready to be implemented. The aim of this study is to compare the stability over time of the concentration of several substances in serum, plasma, and urine stored at two different freezing temperatures (-196°C and -75°C).

Publications and Presentations

At the present time, there are no results or publications coming directly from this grant because we have just begun data collection. However, Dr. Freudenheim has published or has in press research on issues related to this grant using a previously collected data set. In the past year, she presented results regarding alcohol consumption and breast cancer risk at the Annual Meeting of the American Association for Cancer Research (2) and the Annual Meeting of the Society for Epidemiologic Research (3). We found that, although in that data set there was only a weak association between alcohol intake and breast cancer risk, when genetic polymorphisms in the gene for alcohol dehydrogenase, a rate-limiting enzyme in alcohol metabolism, were taken into account, the picture was clearer. We found that pre-menopausal women who were heavier drinkers and who had one of the polymorphisms were at increased risk of breast cancer. Women who were lighter drinkers and/or had the other polymorphism were at lower risk. These findings will be examined in considerably more detail in this new study. (please see appendix for the abstract).

We also have in press a study regarding the relation of lactation history and breast cancer risk. We found in that study that there was a weak protective effect of long duration of breastfeeding (4). (please see appendix for abstract of the manuscript). Other publications originating from the Center for Preventive Medicine on subjects related to the grant are listed in the Reference Section.

CONCLUSIONS

We have just begun data collection for this grant, therefore there are no conclusions to report at this time. Interview of participants is underway.

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2) Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T, Shields PG. Alcohol Intake and Breast Cancer Risk: Effect of Alcohol Metabolism by Alcohol Dehydrogenase 3. Abstract presented at the meeting of the American Association for Cancer Research, San Diego, CA, April 1997.

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8) Muti P, Trevisan M, Modlich F, Krogh V. Use of a Biological Specimen Bank in Epidemiological and Clinical Research. Methodological Issues (under review).

APPENDIX

Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Marshall JR, Graham S, Nemoto T, Shields PG. Breast Cancer, Alcohol and the Alcohol Dehydrogenase 3 Polymorphism. Abstract, American Journal of Epidemiology 1997; 145:S72.

Alcohol dehydrogenase (ADH) is a rate-limiting enzyme in the metabolism of alcohol. The authors examined whether a polymorphism, altering enzyme activity two-fold, modifies the relation of alcohol and breast cancer risk. In a subset of a case-control study, 134 pre- and 181 post-menopausal women with incident, primary, histologically confirmed breast cancer were interviewed and a blood sample obtained. Population controls were frequency matched to cases on age and county (126 pre-, 230 post-menopausal). PCR-RFLP analyses of the ADH polymorphism were performed. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for alcohol based on reported intake in the last 20 years, adjusted for breast cancer risk factors. Among pre-menopausal women, there was increased risk for heavy compared to light drinkers for those with the ADH 1,1 polymorphism (OR 3.5, 95% CI 1.3-9.2) but not ADH 1,2 or 2,2. In a case series analysis with ADH 1,2 and 2,2 combined as the referent, the OR for ADH 1,1 was 1.7 (95% CI 0.8-3.6). For post-menopausal women with ADH 1,1, there was a weak association of alcohol intake with risk (OR 1.4, 95% CI 0.7-3.1) and no alcohol effect for ADH 1,2 or 2,2. The post-menopausal case series analysis did not show a difference in effect of alcohol for the polymorphisms. ADH status may modify the relation of alcohol to breast cancer risk, particularly among pre-menopausal women.

Freudenheim JL, Marshall JR, Vena JE, et al. Lactation History and Breast Cancer Risk. American Journal of Epidemiology (in press).

Lifetime lactation in relation to breast cancer risk was examined in a case-control study in two counties in western New York. Cases were women age 40 and over with incident, primary, histologically-confirmed breast cancer; controls were frequency matched by age and county, selected from New York State driver's license records (<age 65) and Health Care Finance Administration Records (≥ age 65). Included were women with at least one live birth (253 pre- and 367 post-menopausal cases, 266 pre- and 427 post-menopausal controls). Breast cancer risk was very weakly associated with long duration of lactation among pre-menopausal women; odds ratios (OR) and 95% confidence intervals (CI) for at least 20 months lifetime lactation were 0.50 (0.21-1.12). Among post-menopausal women, the protective effect of lactation was restricted to women with first lactation before age 25 (OR 0.67, 95% CI 0.46-0.95). However, age at first birth was highly correlated with age at first lactation. Neither insufficient milk as a reason for not breastfeeding nor having received medication to stop milk flow were associated with increased risk. These findings are in concordance with accumulating evidence that lactation may have a weak protective effect on breast cancer risk.